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# Effect of salts, solvents and buffer on miRNA detection using DNA silver nanocluster (DNA/AgNCs) probes

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## Abstract

MicroRNAs (miRNAs) are small regulatory RNAs (size ~21 nt to ~25 nt) which regulate a variety of important cellular events in plants, animals and single cell eukaryotes. Especially because of their use in diagnostics of human diseases, efforts have been directed towards the invention of a rapid, simple and sequence selective detection method for miRNAs. Recently, we reported an innovative method for the determination of miRNA levels using the red fluorescent properties of DNA/silver nanoclusters (DNA/AgNCs). Our method is based on monitoring the emission drop of a DNA/AgNCs probe in the presence of its specific target miRNA. Accordingly, the accuracy and efficiency of the method relies on the sensitivity of hybridization between the probe and target. To gain specific and robust hybridization between probe and target, we investigated a range of diverse salts, organic solvents, and buffer to optimize target sensing conditions. Under the newly adjusted conditions, the target sensitivity and the formation of emissive DNA/AgNCs probes were significantly improved. Also, fortification of the Tris–acetate buffer with inorganic salts or organic solvents improved the sensitivity of the DNA/AgNC probes. On the basis of these optimizations, the versatility of the DNA/AgNCs-based miRNA detection method can be expanded.

**Keywords:** buffer, silver nanocluster, miRNA detection, salts, solvents

 Online supplementary data available from [stacks.iop.org/Nano/25/045101/mmedia](http://stacks.iop.org/Nano/25/045101/mmedia)

(Some figures may appear in colour only in the online journal)



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## 1. Introduction

The remarkable optical properties of silver nanoclusters (AgNCs) have attracted prominent attention for their potential use as fluorescent labels for microscopic imaging and as sensors for the detection of bio-molecules [1–27]. Numerous

**Table 1.** DNA/AgNC probe sequences. This paper concerns experimental studies of the highly emissive DNA-GG172-12nt-RED. 12nt DNA scaffold (bold), complementary sequences against target miRNA (underlined), and additional GG sequence (italic), DNA-12nt-RED-172GG is a non-fluorescent single stranded probe [24].

Probe	Sequence
DNA-12nt-RED	5'- <b>CCTCCTTCCTCC</b> -3'
DNA-GG172-12nt-RED	5'-GGATGCAGCATCATCAAGATTCT <b>CCTCCTTCCTCC</b> -3'
DNA-12nt-RED-172GG	5'- <b>CCTCCTTCCTCC</b> <i>ATGCAGCATCATCAAGATTCTGG</i> -3'

studies have applied DNA as a scaffold for nanocluster formation, focusing on the photoluminescence aspects of the DNA-templated silver nanoclusters (DNA/AgNCs) for practical applications [1–4, 8, 10, 28–47]. Recently, we have provided proof of principle for a novel miRNA detection method using DNA/AgNC probes, where highly fluorescent DNA/AgNCs probes promptly respond to the presence of target miRNA [40, 48]. Specific examples of this versatile approach include two DNA/AgNCs probes that target plant miR160 (involved in phytohormone regulations) and miR172 (important for flower development). Addition of AgNO<sub>3</sub> and subsequent reduction of the DNA/AgNCs probes with NaBH<sub>4</sub> (DNA/AgNO<sub>3</sub>/NaBH<sub>4</sub> in a 1:17:17 ratio), generates a very bright red fluorescence within an hour. In the presence of their target miRNAs, the glowing red fluorescence of the DNA/AgNCs probes is selectively switched off [40, 48]. However, to translate the method into practical applications, detailed knowledge on the mechanisms underlying the appearance of rapid and strong red fluorescence in the DNA/AgNCs probes is required, and so is a further understanding of the dissipation by target miRNA hybridization. To address this demand, a detailed analysis of the secondary structure of DNA/AgNCs probe designs using deletion fragments proved useful. We showed that secondary structures such as mismatch self-dimer and/or hair-pin in the employed DNA/AgNCs probes, are a crucial determinant for the brightness [48]. To further the understanding of the mechanism of emission drop upon target hybridization, we here closely look into the probe/target hybridization under chemically adjusted conditions, as this is known to be one of the crucial factors for hybridization-based detection of nucleic acids [49–53]. Through detailed buffer/salt or buffer/solvent optimizations on the probe DNA-GG172-12nt-RED, we here report a framework for the improvement of target sensitivity of the presented analytical approach, and provide a benchmark for evaluating the performance of DNA/AgNCs probes. Table 1 depicts the DNA/AgNC probes discussed in this paper, which all rely on a 12-nucleotide scaffold—DNA-12nt-RED—that directs the creation of red emissive AgNCs, and which can be combined with a complementary target specific sequence.

## 2. Experimental section

### 2.1. Materials and reagents

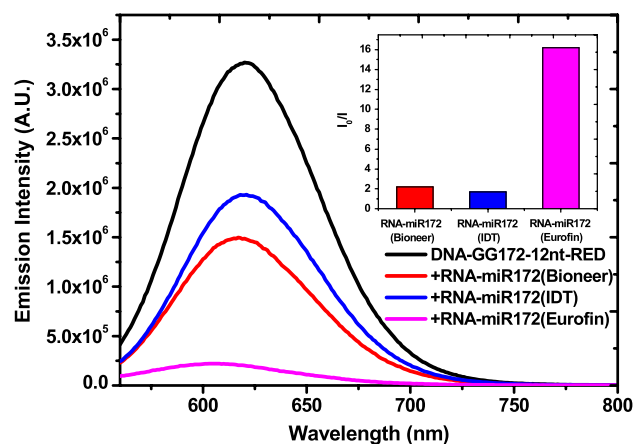
DNA probes and desalted miRNA targets were obtained from three different commercial suppliers: IDT (Integrated DNA Technologies, BVBA, Interleuvenlaan 12A, 3001

Leuven, Belgium), Bioneer (Bioneer Corporation, 8-11 Munpyeongseo-ro, Daedeok-gu, Daejeon 306-22, Korea), and Eurofin (Eurofins MWG Synthesis GmbH, Anzinger Straße 7a, D-8556 Ebersberg, Germany). The synthesis of emissive AgNCs was carried out using AgNO<sub>3</sub> (99.9999%) and NaBH<sub>4</sub> (99.99%) from Sigma Aldrich. Tris–Acetate buffer (pH7, 0.5 M) was prepared with TRIZMA® acetate salt (≥99.0%, from Sigma Aldrich) in pure Milli-Q water (18.2M MΩ cm). The stock solution of Tris–HCl buffer (pH7, 0.5 M) was prepared by pH adjustment using HCl. The high purity salts (sodium nitrate, potassium nitrate, magnesium acetate, magnesium nitrate, magnesium sulfate) and solvents were purchased from discrete manufacturers. Methanol, isopropanol and 1-butanol were purchased from discrete manufacturers and were at least 99% pure. 96% ethanol stock solution was used to check for the effect of ethanol on the formation of DNA/AgNCs. All solvents were diluted to 10% with water and were thoroughly mixed before adding them to the buffered DNA solution.

**2.1.1. Synthesis of DNA/AgNCs probes and target miRNA detection.** The DNA and miRNA sequences used in the publication are described in table 1. To make fluorescent AgNCs, we incubate the DNA-GG172-12nt-RED probe at 25 °C for 20 min in the given concentrations of Tris–acetate buffer with or without salts, followed by an addition of AgNO<sub>3</sub> (250 μM) and NaBH<sub>4</sub> (250 μM), (1:17:17) to a final volume (50 μl). For the miRNA detection assay, we added a fixed amount of target, RNA-miR172 (15 μM), to the DNA-GG172-12nt-RED (15 μM) at the given concentrations of Tris–acetate buffer with or without salts (the designated concentrations in figure legends are the actual concentrations after 10 times dilution for measurement) and incubated for 15 min at 25 °C after denaturation. Then, AgNO<sub>3</sub> (250 μM) and NaBH<sub>4</sub> (250 μM) were added to the RNA/DNA mixtures to a final volume (50 μl). All the DNA/AgNCs were incubated for 1 h at 25 °C and diluted with 450 μl of distilled water before measurement by a fluorimeter (Horiba Jobin Yvon, Fluoromax-4) in a 10 mm disposable cuvette. We here designated the concentrations of nucleic acids, buffer and salts in the final volume for measurements (500 μl). For AgNC formation in RNA–miRNAs (15 μM in 50 μl), we added AgNO<sub>3</sub> and NaBH<sub>4</sub> in a 1:17:17 ratio to a final volume (50 μl). Before measuring the fluorescence, the volume was increased from 50 to 500 μl for all RNA–miRNAs.

## 3. Results and discussion

The concept applied for miRNA detection is to use a DNA scaffold (DNA-12nt-Red) tethered to a DNA

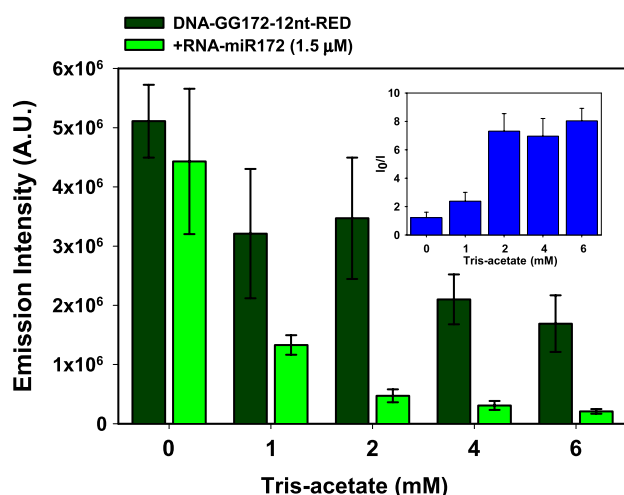


**Figure 1.** Fluorescence intensity of the DNA-GG172-12nt-RED probe ( $1.5 \mu\text{M}$ ) in the presence of the RNA-miR172 target ( $1.5 \mu\text{M}$ ) following excitation at 540 nm. The emission of DNA-GG172-12nt-RED in the absence of RNA-miR172 (black curve), in the presence of RNA-miR172 provided by Bioneer (red curve), in the presence of RNA-miR172 provided by IDT (blue curve) or in the presence of RNA-miR172 from Eurofin (pink curve). Inset shows  $I_0/I$  values of DNA-GG172-12nt-RED probe in the presence of RNA-miR172 provided by different suppliers: Bioneer (red bar), IDT (blue bar) and Eurofin (pink bar).

sequence complementary to the target miRNA for silver encapsulation [40]. Basically our method is a turn-off system, which is dependent on two processes: (1) the creation of a highly fluorescent AgNC species in solution, and (2) the emission drop following target miRNA hybridization [40]. However, in the subsequent design of bright DNA/AgNCs probes, we encountered the problem that some DNA probes were barely emissive. To circumvent the problem, a series of particular experiments on a highly emissive DNA/AgNCs probe (DNA-12nt-Red-160) were commenced [48]. Through the study, we found that the rapid creation of highly bright red AgNCs is attributed to the secondary structures of the DNA-12nt-Red-160 probe and showed that the formation of mismatch self-dimers are a good indicator for highly emissive probes [48]. During further testing of various DNA/AgNCs probes such as DNA-GG172-12nt-RED, we observed an inconsistency in the target miRNA detection. Synthesized miRNAs from some commercial suppliers were not efficiently recognized in our highly stringent reaction conditions (milli-Q water). For instance, the RNA-miR172s ( $1.5 \mu\text{M}$ ) from two other companies (IDT and Bioneer) did not affect the emission of the DNA probe whereas RNA-miR172 ( $1.5 \mu\text{M}$ ) from Eurofin triggered a dramatic drop of emission when it was added to the equivalent amount of DNA-GG172-12nt-RED probe ( $1.5 \mu\text{M}$ ) (figure 1). The RNA-miR172 from Eurofin leads to an  $I_0/I$  ratio of 16, where  $I_0$  is the emission intensity value without and  $I$  the intensity with the target RNA-miR172 present. As can be seen in the inset of figure 1, the RNA-miR172s from IDT and Bioneer both have  $I_0/I$  ratios of less than two. To address this inconsistency, we here present a systematic study on the optimization of the reaction conditions for the creation of highly emissive DNA/AgNCs probes with high sensitivity.

Nucleic acid secondary structure and hybridization is highly dependent on physical and chemical factors such as temperature, pH, and the ionic composition of the solutions [49, 50, 52–56], and any nucleic acid in solution will be accompanied by counter ions of some kind. We therefore speculated that the observations of non-functional RNA-miR172s might be due to differences in the electrolytes carried by the RNA products. We tested three different types of RNA-miR172 products (desalted, HPLC and PAGE-purification) from IDT and could conclude that all were invalid in our high stringency condition (data not shown). In DNA/RNA duplex formation, Tris–Cl buffers are often used to maintain an adequate pH and as a source of the monovalent cation ( $\text{TrisH}^+$ ) for the annealing between two strands of nucleic acids [53]. However, the high concentration of chloride ions in a Tris–Cl buffer can sequester silver ions, which obstructs the creation of emissive AgNCs due to solubilized or even precipitated AgCl or the formation of coordination species such as the dichloridoargentate (I) ion. Indeed, practically all anionic buffer components will have some affinity for binding silver ions. Tris itself also has a silver ion binding capability when the amino group is deprotonated, the  $\text{TrisH}^+$   $\text{pK}_a$  being 8.16 at  $25^\circ\text{C}$  [57]. Here, we tested both Tris–Cl buffer and a potential substitute, Tris–acetate buffer. The creation of silver nanoclusters in DNA-GG172-12nt-RED probe was performed in a solution containing Tris–Cl (2 mM, pH7.0) or Tris–acetate (2 mM, pH7.0). Indeed, the presence of Tris–Cl buffer (2 mM) severely impeded the generation of bright red emission, while the emission intensity of the probe in Tris–acetate (2 mM) was only  $\sim 30\%$  reduced compared to control fluorescence experiments in milli-Q water, suggesting that Tris–acetate is a suitable buffer for use in the creation of highly emissive AgNCs (see supporting information figure 1 available at [stacks.iop.org/Nano/25/045101/mmedia](http://stacks.iop.org/Nano/25/045101/mmedia)). Accordingly, we mainly focused on optimizing the concentrations of Tris–acetate buffer, either to maintain the creation of highly emissive AgNCs or to enhance probe/target hybridization. As shown in figure 2(A), with excitation at 540 nm, the fluorescence of DNA-GG172-12nt-RED obtained at 620 nm is gradually decreased by an increasing concentration of Tris–acetate (1–6 mM). At 1 and 2 mM Tris–acetate, the probe displays  $\sim 70\%$  of the emission intensity compared to the water control. The red fluorescence of the probe further lowered to the range  $\sim 50\%$  to  $\sim 30\%$  of the water control at higher concentrations of Tris–acetate (4 and 6 mM) (figure 2(A)). Thus, the tested concentrations of Tris–acetate decrease the fluorescence without the addition of RNA-miR172. However, the adverse effect of Tris–acetate is acceptable, because the lowest fluorescence of DNA-GG172-12nt-RED at 6 mM Tris–acetate is still 80 times brighter than DNA-12nt-RED, and is thus still sufficient to detect the target presence by the emission drop. The short scaffold DNA-12nt-RED (table 1) generates a baseline intensity of AgNCs ( $2 \times 10^{-4}$ ) in an hour and is not affected further by the addition of any miRNA sequences. To set a standard of emission intensity, we designated 50-fold higher emission ( $1 \times 10^{-6}$ ) than the baseline intensity ( $2 \times 10^{-4}$ ) as the minimum requirement





**Figure 2.** Fluorescence profile of the DNA-GG172-12nt-RED probe at different concentrations of Tris-acetate buffer (1, 2, 4 and 6 mM) in the absence (olive bar) or presence of the target miRNA (green bar). Inset shows the  $I_0/I$  ratio of the fluorescence intensity of DNA-GG172-12nt-RED probe. Data were collected as average measurements with  $\pm$  standard deviations (SD;  $n = 15$ ).

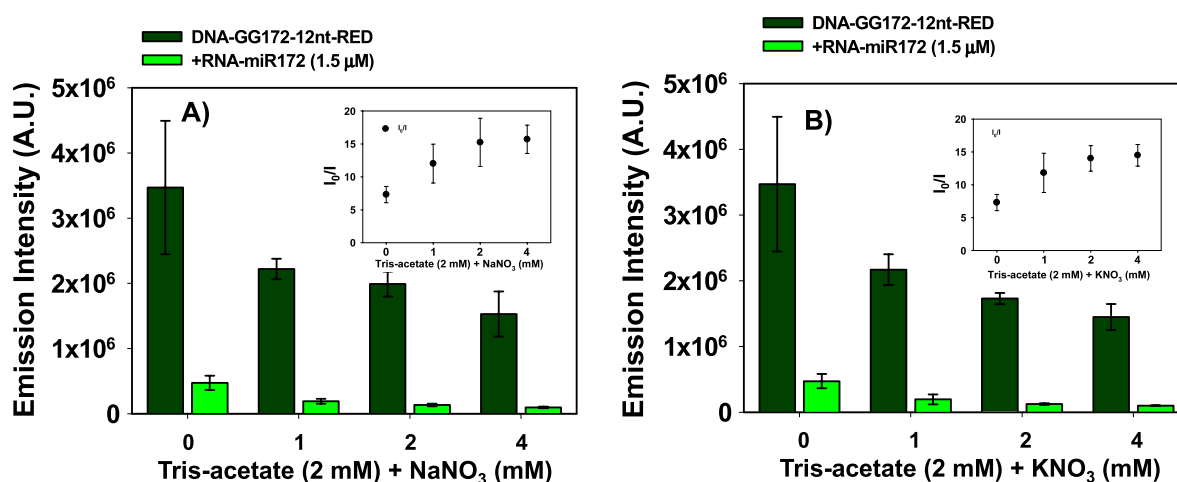
of emission intensity for miRNA detection by fluorescence drop. We verified that the pattern of full spectral scans obtained with Tris-acetate buffer are not changed compared to that observed without buffer (a full spectral scan of the emission as a function of excitation wavelength for DNA-GG172-12nt-RED is shown in supporting information figure 1 available at [stacks.iop.org/Nano/25/045101/mmedia](http://stacks.iop.org/Nano/25/045101/mmedia)). DNA-GG172-12nt-RED shows a maximum fluorescence at 620 nm when excited at 540 nm.

Next, we investigated our major question whether DNA-GG172-12nt-RED efficiently recognizes the presence of RNA-miR172 in Tris-acetate buffer adjusted conditions. Experiments were conducted by annealing DNA-GG172-12nt-RED and RNA-miR172 in the absence or presence of Tris-acetate buffer, and subsequently adding AgNO<sub>3</sub> and reducing with NaBH<sub>4</sub>. For these and subsequent experiments synthesized miRNAs supplied by IDT were used as representatives of miRNAs that are unresponsive under high stringency conditions. Without Tris-acetate buffer, DNA-GG172-12nt-RED maintained its initial emission intensity even in the presence of RNA-miR172 (1.5  $\mu$ M) with an  $I_0/I$  factor of 1.1 (figure 2). When adding an increasing concentration of Tris-acetate buffer to the DNA-GG172-12nt-RED/RNA-miR172 annealed mixtures, the bright red emission of DNA-GG172-12nt-RED notably dropped at 2 mM, 4 mM and 6 mM to average  $I_0/I$  ratios of 7, 7, and 8, respectively (see figure 2, inset). Despite the  $I_0/I$  factor being highest at 6 mM, the 2 mM Tris-acetate condition is considered an optimized point for the DNA-GG172-12nt-RED probe because the emission intensity of the control is twice as high as that of the control at 6 mM. In fact, the  $I_0/I$  factor of 7 at 2 mM Tris-acetate in the presence of RNA-miR172 is a remarkable improvement. Taken together, we conclude that the supplement of Tris-acetate buffer is efficacious but has to be confined to 2 mM to maintain the

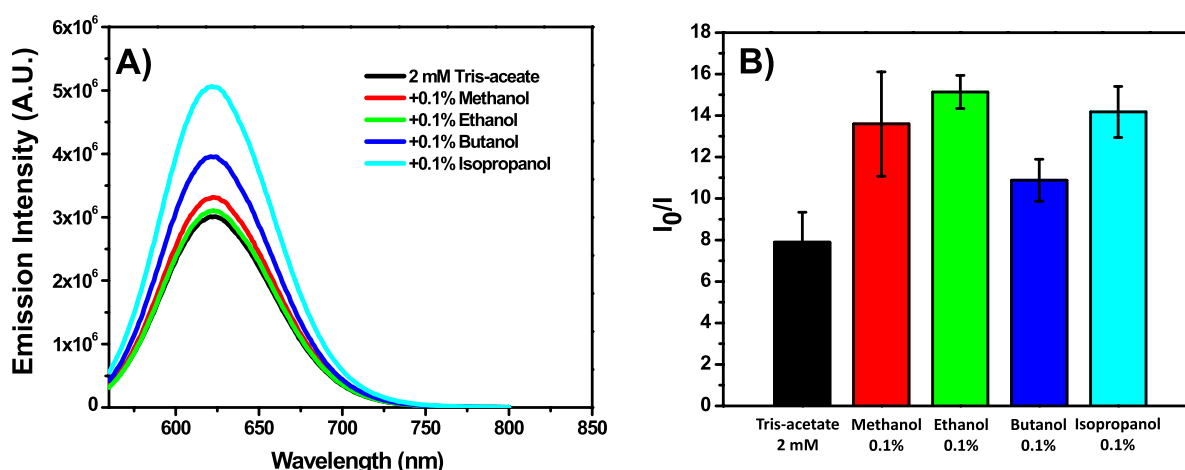
fluorescence of DNA probes above the minimal standard emission intensity.

Low stringency conditions favor target/probe annealing, thus Tris-acetate buffers fortified by metal ions such as Na<sup>+</sup> and Mg<sup>2+</sup> play an essential role in complementary hybridization for polyanionic nucleic acid species through specific electrostatic screening [50, 54–56]. A recent study confirmed that Na<sup>+</sup> is an important electrolyte for DNA target recognition by DNA/AgNCs probes [54]. Therefore, we examined whether the addition of a specific salt to Tris-acetate buffer further facilitates DNA probe/miRNA hybridization and spectroscopic response. First, we tested several electrolyte candidates, such as sodium nitrate, potassium nitrate, magnesium acetate, magnesium nitrate and magnesium sulfate, to select whichever works best with the least impeding effect on the evolution of bright red AgNCs. Indeed, some of the salts, such as magnesium acetate, magnesium sulfate, and magnesium nitrate, conspicuously compromised the brightness of DNA-GG172-12nt-RED probe, while sodium nitrate and potassium nitrate did not (see supporting information figure 2 available at [stacks.iop.org/Nano/25/045101/mmedia](http://stacks.iop.org/Nano/25/045101/mmedia)). Although Ma *et al*, showed the positive role of magnesium ions in the creation of emissive AgNCs [58], the treatment with magnesium ions under our reaction conditions interrupts the formation of bright red AgNCs. On the basis of this test, we investigated the effect of NaNO<sub>3</sub> or KNO<sub>3</sub> combined Tris-acetate buffer on DNA probe/target hybridizations. To maintain the brightness of probes and to improve the detection sensitivity, we fixed the concentration of Tris-acetate buffer at 2 mM and optimized the concentration of the salts. In the given concentrations (1, 2 and 4 mM) of NaNO<sub>3</sub>, the target sensitivity of DNA-GG172-12nt-RED probe was significantly enhanced to  $\sim 1.6$  ( $I_0/I = 11$ ),  $\sim 2.1$  ( $I_0/I = 15.3$ ), and  $\sim 2.1$  ( $I_0/I = 14.5$ ) times, respectively (see figure 3(A)). Furthermore, a series with varied KNO<sub>3</sub> addition (concentrations of 1, 2 and 4 mM), was similarly highly effective on the  $I_0/I$  ratio ( $\sim 11$ ,  $\sim 14$ , and  $\sim 14$ , respectively), suggesting that both salts are good electrolytes for enhancing the target sensitivity of the DNA-GG172-12nt-RED probe (figure 3(B)). Nevertheless increasing concentrations of NaNO<sub>3</sub> or KNO<sub>3</sub> further lower the initial emission of control. Overall, NaNO<sub>3</sub> or KNO<sub>3</sub> addition to a Tris-acetate buffer is a fairly suitable buffer composition for our method because of two aspects: firstly, as we mentioned above, the lowered emission intensities of the probe ( $2 \times 10^{-6}$  to  $1 \times 10^{-6}$ ) are still high enough for miRNA detection by a drop in the emission intensity. Secondly, the target sensitivity is improved by a factor of two by addition of NaNO<sub>3</sub> or KNO<sub>3</sub>, which meets the principal demand of our method.

Organic solvents have been widely used to precipitate nucleic acids in aqueous solution. Organic solvents screen the electrostatic repulsion between nucleic acids, promoting the condensation and further precipitations of nucleic acids. The dielectric constant of solvents such as alcohols, acetone and ethylene glycol is a good indicator for the effect of these solvents on the conformational behavior of DNA structures in aqueous solution. When the dielectric



**Figure 3.** (A) Maximum fluorescence emission of the DNA-GG172-12nt-RED probe (1.5  $\mu$ M) in Tris-acetate buffer (2 mM) augmented with increasing amounts of NaNO<sub>3</sub> (1, 2, and 4 mM) in the absence (olive bars) or presence (green bars) of the target RNA-miR172. Inset displays the effect of sodium nitrate on  $I_0/I$  values. (B) Maximum fluorescence emission of the DNA-GG172-12nt-RED probe (1.5  $\mu$ M) in Tris-acetate buffer (2 mM) augmented with increasing amounts of KNO<sub>3</sub> (1, 2, and 4 mM) in the absence (olive bars) or presence (green bars) of the target RNA-miR172. Inset displays the effect of sodium nitrate on  $I_0/I$  values. Data were collected as average measurements with  $\pm$ standard deviations (SD;  $n = 15$ ).



**Figure 4.** (A) Maximum emission spectrum of the DNA-GG172-12nt-RED probe (1.5  $\mu$ M) in Tris-acetate buffer (2 mM) augmented with various solvents; without solvents (black curve), 0.1% methanol (red curve), 0.1% ethanol (green curve), 0.1% butanol (blue curve), and 0.1% isopropanol (cyan curve). (B) The effect of solvents on  $I_0/I$  values in the presence of 1.5  $\mu$ M RNA-miR172. Data were collected as average measurements with  $\pm$ standard deviations (SD;  $n = 10$ ).

permittivity constant in the solvent system is lowered, the influence of ion-ion correlation is increased, which induces the DNA compactions [59]. Although the integrity and dynamics of DNA structures with silver clusters in organic solvents have not been fully elucidated, we examined the effect of organic solvents on the creation of highly emissive DNA/AgNCs probes inspired by recent reports suggesting the effect of organic solvents on poly-methacrylic acid (PMAA)-encapsulated AgNCs. The emission wavelength of PMAA-encapsulated AgNC was notably changed in water-methanol mixtures [60]. On the other hand, Xu *et al* reported that the fluorescence intensity of the PMAA-encapsulated AgNC can be enhanced without any spectral shifts of AgNCs in solvents [61]. So far, to the best of our knowledge, the effect of organic solvents on

DNA/AgNCs has not been investigated. Thus, we examined alcoholic solvents such as ethanol, methanol, butanol or isopropanol as a supplement for Tris-acetate buffer. Prior to the creation of silver nanoclusters, we briefly incubated the DNA template, DNA-GG172-12nt-RED in various organic solvents, ethanol, methanol, butanol, and isopropanol. As shown in figure 4(A), the addition of 0.1% isopropanol dramatically enhanced the fluorescence of the DNA/AgNCs probe. Also, the fluorescence of DNA-GG172-12nt-RED was slightly enhanced in butanol (0.1%) by a factor of around 1.2. In the case of ethanol (0.1%) or methanol (0.1%) treatment we could not observe the intensity enhancement of fluorescence. The emission wavelengths of the DNA-GG172-12nt-RED probe were not changed by the addition of solvents, unlike previously reports that the emission spectra of

PMAA- or methacrylic acid (MAA) polymer-encapsulated AgNCs are dramatically shifted in organic solvents (see supplementary information figure 2–6 available at [stacks.iop.org/Nano/25/045101/mmedia](http://stacks.iop.org/Nano/25/045101/mmedia)) [60–63]. The chemical mechanism that differentiates the effect of each solvent addition on AgNCs formation with DNA or organic polymer encapsulation is not known, but the structural stability of a given scaffold is important. To rule out the direct influence of solvents on AgNCs, we tested the non-structured DNA-12nt-RED-172GG, which does not form highly emissive AgNCs (table 1). As shown in supplementary information figure 7 (available at [stacks.iop.org/Nano/25/045101/mmedia](http://stacks.iop.org/Nano/25/045101/mmedia)), spectral shifts or emission enhancement were not observed, suggesting the importance of the structures in scaffolds. Next, we tested the target sensitivity of the DNA-GG172-12nt-RED probe in the mixed organic solvent Tris–acetate buffer. Interestingly, by the addition of all tested solvents (methanol, ethanol, butanol, and isopropanol), the target sensitivity of DNA-GG172-12nt-RED probe was significantly increased by  $\sim 1.9$  ( $I_0/I = 13.5$ ),  $\sim 2.1$  ( $I_0/I = 15$ ), and  $\sim 1.5$  ( $I_0/I = 10.3$ ), and  $\sim 2$  ( $I_0/I = 14$ ) times, respectively (figure 4(B)). The treatment of isopropanol effectively increased both the emission intensity and target sensitivity of DNA probes while methanol and ethanol enhanced only the target sensitivity. The origin of the differentiated effects of each solvent is not clearly understood, but could be related to the different chemical features amongst the polar protic solvents, such as dielectric constant, refractive index, and number of non-polar C–H bonds. As we previously reported [24], the rapid evolution of bright emission in a DNA probe is largely dependent on its secondary structures, such as hair-pin and/or self-dimer formation. Therefore, the target sensitivity can be dependent on the strength of target hybridization, which can open the hair-pin and/or separate the self-dimer, consequently leading to an emission drop. Here, we have shown that the addition of salt or co-solvents with Tris–acetate buffer can dramatically improve the target sensitivity of a DNA/AgNC probe. This study implies that the balance between the structural stability of a DNA/AgNCs probe versus its target accessibility can be modulated through detailed buffer optimization. These prospects will be further adopted to rationally design DNA/AgNCs probes against a variety of miRNA targets in plants and in humans as well as in other biological contexts.

#### 4. Conclusion

To ensure a highly reliable and robust method utilizing DNA/AgNCs probes, a high emission and a simultaneous high target sensitivity are two indispensable optimization criteria. In our probe design, high emission relies on the secondary structures of the DNA/AgNCs probes. In this study, we have furthermore shown that the target sensitivity of the DNA-GG172-12nt-RED probe can be dramatically enhanced by Tris–acetate buffer and further improved by the addition of salts or organic co-solvents. The high fluorescence coupled with reliable sensitivity using the combined buffer system is an important step towards optical miRNA detection in more complex situations.

#### 5. Associated content

Procedure details for the creation of emissive AgNCs and buffer optimization. Full spectra scan of DNA-GG172-12nt-RED in water or in Tris–Cl buffer or in Tris–acetate buffer. Effect of various salts on the formation of emissive AgNCs in DNA-GG172-12nt-RED.

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